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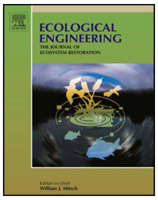

Evaluation of substrates for constructing beds for the marine macrophyte *Zostera marina* L.

Wataru Nishijima[a,*], Yoichi Nakano[b], Amelia B. Hizon-Fradejas[c], Satoshi Nakai[d]

[a] Environmental Research and Management Center, Hiroshima University, 1-5-3, Kagamiyama, Higashihiroshima, Hiroshima 739-8513, Japan
[b] Department of Chemical and Biological Engineering, Ube National College of Technology, 2-14-1, Tokiwadai, Ube, Yamaguchi 755-8555, Japan
[c] Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Fukuoka 812-8581, Japan
[d] Graduate School of Engineering, Hiroshima University, 1-4-1, Kagamiyama, Higashihiroshima, Hiroshima 739-8527, Japan

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ABSTRACT

The selection of substrate material is a critical factor for successful construction of eelgrass beds. We evaluated the effects of different proportions of silt and clay particles ($<75\,\mu m$) in sediment on the anchorage of eelgrass roots using natural eelgrass bed sediment and mountain sand as basal materials, and silt and clay particles separated from natural sediment, dredged sediment or fly ash as the silt and clay component. We evaluated the anchorage of plants by their resistance to an uprooting force. Silt and clay particles were important to the anchorage of eelgrass roots. Complete removal of silt and clay particles from natural eelgrass bed sediment clearly reduced the uprooting resistance by about half. The optimal silt and clay content for eelgrass root anchorage was 15–20%. The addition of dredged sediment or fly ash to mountain sand enhanced the uprooting resistance of eelgrass plants.

1. Introduction

Marine seagrass beds are widely distributed and are characteristic of estuarine and marine coastal environments. These submerged and emergent meadows provide a good habitat for a wide variety of fishes and macrobenthos and support high productivity and biodiversity (Blanchet et al., 2004; Edgar and Shaw, 1995a,b,c; Hicks, 1986). They also enhance sediment stabilization and dampen wave energy (Gambi et al., 1990; Hendriks et al., 2008) resulting in a stable environment for marine organisms.

Eelgrass (*Zostera marina* L.) is the most popular seagrass in the enclosed sea of Japan and has rapidly declined during economic development. In the Seto Inland Sea, the largest enclosed sea in Japan, the area of eelgrass beds decreased from 22,635 ha in 1960 to 5574 ha in 1971 (Minister of the Environment, 2013). Many studies indicate that the distribution and abundance of seagrasses in temperate coastal zones is mainly controlled by light availability (Backman and Barilotti, 1976; Hauxwell et al., 2006; Koch and Beer, 1996). Anthropogenic activities have significant negative impacts on the survival of seagrass, *Zostera marina* L., for example, through decreases in water transparency and deposition of suspended solids on submerged leaves resulting from increases in turbidity and the growth of algae (Tamaki et al., 2002). In addition to the negative effects of decreased light availability, eelgrass beds are directly destroyed by dredging, development and reclamation of coastal zones (Erftemeijer and Lewis, 2006; Komatsu, 1997).

Worldwide, lost seagrass beds are being restored and new ones are being created (de Jonge and de Jong, 2002; de Jonge et al., 2000; Sogard, 1989; Sugimoto et al., 2008; Zimmerman et al., 1995). In Japan, The Nature Conservation Bureau of the Ministry of the Environment is implementing various projects in cooperation with other related Ministries and agencies to restore ecologically important environments including seagrass beds that have been lost in the past. Seagrass habitat restoration can include mounding of the sediment to improve light conditions on the beds, transplanting seeds or adult shoots, or combinations of these (Bastyan and Cambridge, 2008; Davis and Short, 1997; Orth et al., 1999; Park and Lee, 2007; Sugimoto et al., 2008; van Katwijk et al., 1998). The mounding option is especially effective in areas where seagrass beds existed in the past and were lost though a decline in water transparency due to eutrophication. The choice of materials for the mounding is crucial for the survival of both transplanted and naturally invading eelgrass.

The physical properties of sediment mainly affect germination, root penetration and growth in seagrass (Handley and Davy, 2002). The nutrient content of sediment supporting eelgrass also affects

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[*] Corresponding author. Fax: +81 82 424 6199.
E-mail address: wataru@hiroshima-u.ac.jp (W. Nishijima).

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eelgrass characteristics such as shoot height, leaf area and growth, biomass and chemical composition (Road, 1987; Short, 1983). Handley and Davy (2002) suggested that the size of substrate particles and closeness of packing were likely to affect the rooting of aquatic plants. Li et al. (2012) showed that the freshwater eelgrass *Vallisneria natans* grown in silt and clay had greater height, more ramets and leaves, and greater biomass accumulation compared to *V. natans* grown in pebble and gravel substrates. Healthy eelgrass beds are found in substrates with wide range of silt and clay content (1–56%; Imao and Fushimi, 1985; Keser et al., 2003; Koch, 2001; Laugier et al., 1999; Tamaki et al., 2002). Although, a high silt–clay content can create a reducing environment due to decreased exchange of pore water with the water column (Huettel and Rusch, 2000; Koch, 2001). The reducing conditions and sulfides often observed in sediments rich in organic matter inhibit growth and increase mortality in seagrasses (Calleja et al., 2007; Holmer and Nielsen, 1997; Terrados et al., 1999).

At present, it is much difficult to obtain adequate materials for sediment mounding from natural environments. Sea sand had been often used as the base material in most of the coastal restoration projects in Japan (Hosokawa, 1997; Lee et al., 1998). However, the excavation of sea sand has been forbidden in most areas of the Seto Inland Sea because of the environmental impacts of the excavation itself (Takeoka, 2002). Therefore, it is necessary to develop new materials to restore or create eelgrass beds.

Some sand-like materials have been considered and evaluated for eelgrass substrate including; blast furnace slag produced as a byproduct of steel making (Hizon-Fradejas et al., 2009a,b), mountain sand (MS; Hizon-Fradejas et al., 2010) and granulated coal fly ash (FA), produced from thermal power stations (Asaoka et al., 2009). Approximately 24 million tonnes of blast furnace slag and 10 million tonnes of FA are produced each year in Japan (Nippon Slag Association, 2011; Tanaka et al., 2010), and are thus available in sufficient quantity should they prove desirable materials for eelgrass bed restoration. Dredged sediment (DS) was also considered and evaluated as a silt–clay source (Hizon-Fradejas et al., 2009b, 2010).

The ability of a material to hold eelgrass and maintain the stability of the sediment against disturbance by waves and flows are also important factors in the selection of materials for mounding. Several studies have suggested that the dynamic forces produced by waves affect the physical stability of the organisms (Denny et al., 1998; Gaylord et al., 2001; Utter and Denny, 1996). In our previous study with slag substrates, we found that the force needed to uproot eelgrass plants was higher for transplants in fine particles than in coarse particles (Hizon-Fradejas et al., 2009b). The purpose of this study is to evaluate the effect of mixtures of MS as the basal material and DS or FA as the silt–clay component on the anchorage ability of eelgrass roots.

2. Materials and methods

2.1. Substrates

The importance of fine particles in holding plants in sediments using both natural eelgrass bed sediment (NES) and two modified NESs was first evaluated. NES was collected from a healthy eelgrass bed at the town of Yoshina on the Seto Inland Sea, Japan. Two modified NES without fine particles were prepared by sieving through 75-μm or 850-μm mesh sieves. To make modified NES with proportions of silt–clay particles (<75-μm) ranging from 0% to 40%, the silt–clay separated from the NES was mixed back into the 75-μm mesh sieved NES. Table 1 shows the grain-size distributions of the NES and the modified NES used in the study.

The effect of mixtures of MS as the basal material and DS or FA as the silt–clay component on the anchorage ability of eelgrass roots was then evaluated. As an alternative to sea sand, we used commercially obtained MS after first sieving it through a 75-μm mesh sieve. As silt–clay sources, we used DS excavated from Ago Bay, Japan and FA from a Japanese coal thermal power plant. Samples prepared include MS without DS or FA added, four kinds of mixtures of MS with 5%, 10%, 15% and 20% of DS, and four kinds of mixtures of MS with 5%, 10%, 15% and 20% of FA.

Grain-size distributions were determined according to the Japanese Industrial Standard (JIS) test method for particle–size distribution of soils (Method A 1204; JIS, 2000), wherein sediment to be analyzed were pretreated with 30% hydrogen peroxide and then wet sieved through 75-, 106-, 250-, 425-, 850- and 2000-μm mesh sieves. The organic matter content of each silt–clay source was determined as ignition loss according to the JIS test method for ignition loss of soils (Method A 1226; JIS, 2009).

2.2. Experimental setup

Healthy eelgrasses with shoots 50–60 cm in length were collected for laboratory experiments from the eelgrass bed at Yoshina where NES was also collected. The plants were quickly transported to the laboratory where they were cleaned and planted in sediment collected from the same beds where they had grown. Plants were acclimatized to the laboratory environment in a 2-m^3 tank filled with filtered sea water (salinity 30 psu) from the Fisheries Experiment Station of Yamaguchi, Japan. The acclimatization was carried out at 20 °C with a light regime of 12 h light and 12 h dark and an irradiance of 180–210 μmol-photons $m^{-2} s^{-1}$. Plants weakening or withering during the acclimatization period were not used for experiments.

After acclimatization, leaves and rhizome nodes were removed from each plant, leaving only the three youngest leaves and three nodes of the rhizome. The shoots of each plant were trimmed to approximately 50 cm in length. Three such plants were then planted in a pot and three pots were prepared for each substrate (a total of nine plants per substrate). The plants in the pots were incubated in one large tank under the same conditions during the acclimatization.

Table 1
Grain-size distributions of natural eelgrass bed sediment (NES) and modified NES. All values are percentages by weight.

Grain-size (μm)	NES (%)	NES excluding particles <75 μm (%)	NES excluding particles <850 μm (%)
>2000	3.2	2.6	18.7
850–2000	12.9	14.1	66.6
425–850	21.7	27.5	10.5
250–425	17.9	19.9	1.0
106–250	15.2	23.9	0.6
75–106	3.9	4.0	0.1
<75	25.1	8.0	2.5